Exploring the regulatory impact of head-to-head oriented lncRNAs on germ layer-specific transcription factors

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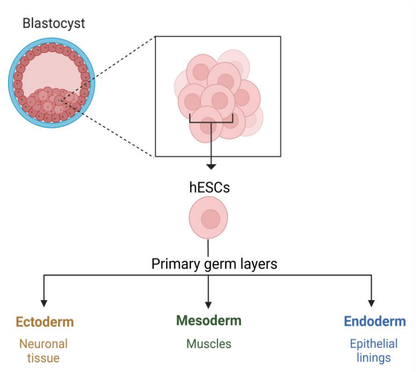
Abstract

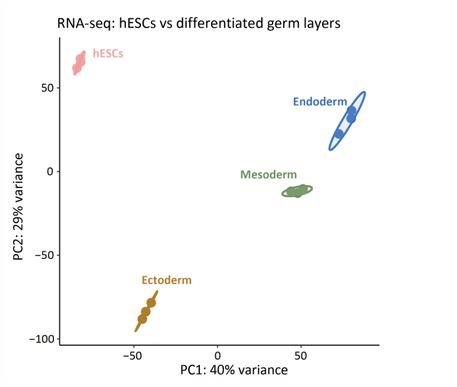
Embryo formation relies on precise combinatorial regulation by transcription factors (TFs) and epigenetic mechanisms which essential to establish cell identity during embryogenesis. This tight regulation is evident in human embryonic stem cells (hESCs), which originate from the early embryo, have the unique ability to differentiate into the three primary germ layers - endoderm, mesoderm, and ectoderm that eventually give rise to all tissues and organs in the body.

Recent studies have revealed that long non-coding RNAs (lncRNAs) play a crucial role in "fine-tuning" gene expression during differentiation in a cell-type-specific manner. We have recently identified lncRNAs that shares promoter regions with TFs in a head-to-head (H2H) orientation. Despite hundreds of H2H pairs in the human genome, only a few have been functionally characterized. We propose that genome-wide H2H-oriented lncRNAs regulate developmental processes by controlling core TF expression, potentially in concert with epigenetic regulators. To identify H2H lncRNAs with novel regulatory functions, we differentiated hESCs and applied high-throughput approaches to profile their expression, protein interactions and chromatin accessibility. Integrating these results through bioinformatics analysis, we generated a list of potentially functional candidates, among which we identified LHX5-AS1, a lncRNA with high expression and selective protein interactions in the ectoderm, showing stage-specific expression like its neighboring TF LHX5, a key regulator of brain development.

We hypothesize that genome-wide H2H-oriented lncRNAs regulate core transcription factors essential for developmental processes through unique and diverse mechanisms. Specifically, we hypothesize that LHX5-AS1 plays a pivotal role in early differentiation by modulating LHX5 expression during ectodermal development.

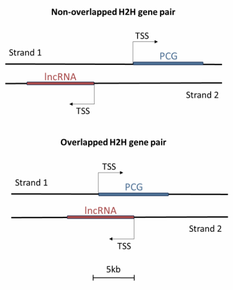
Human embryonic stem cell (hESCs) differentiation to three primary germ layers

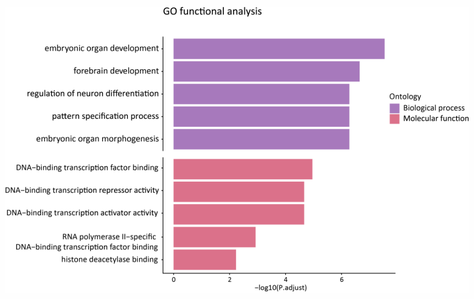


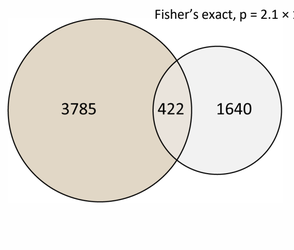


- hESCs and each germ layer exhibits distinct expression patterns.

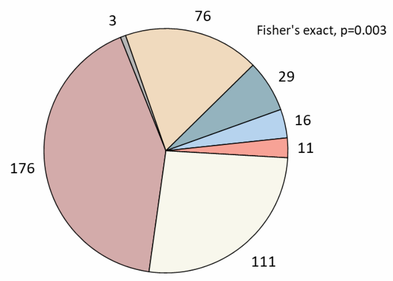
Screening H2H protein-coding genes (PCG)-lncRNA pairs in human genome







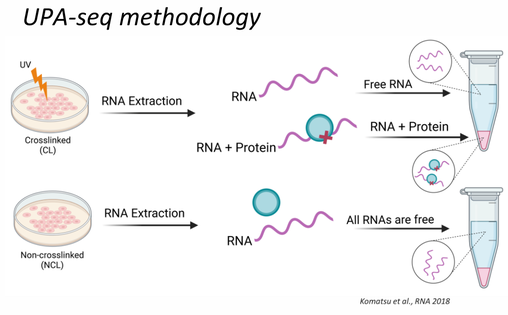
- Protein-coding genes in H2H orientation with lncRNAs are significantly associated with embryonic development and transcription regulation.
- 26% of the genes encoding for transcription factors in the human genome are positioned in H2H orientation with lncRNAs.

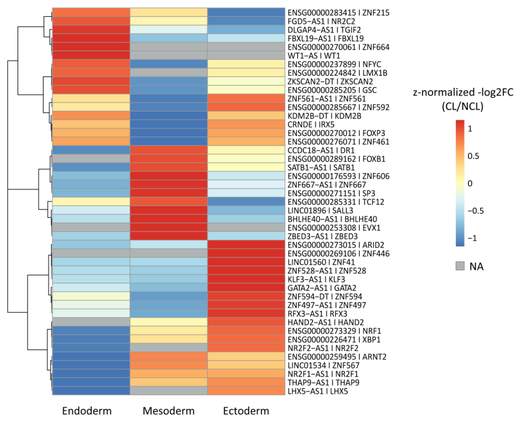


- The Homeodomain family is significantly enriched among H2H TFs compared to its overall abundance in the human genome.

Identifying protein-binding H2H lncRNAs linked to TF biomarkers

UPA-seq methodology

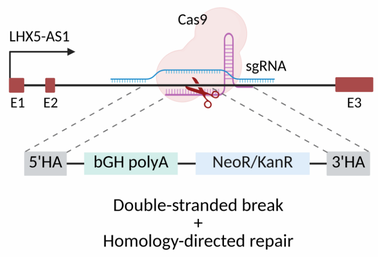




- We revealed highly significant protein-binding lncRNAs H2H-oriented with TFs.

Current and future research

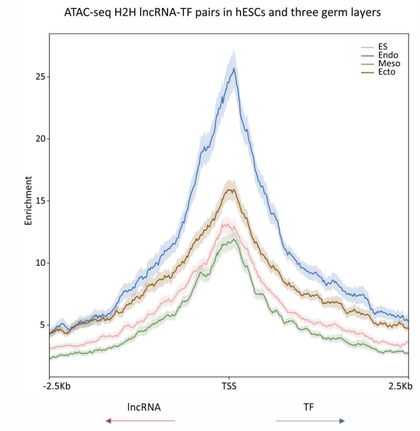
1. We employed CRISPR-mediated knock-in to disrupt LHX5-AS1 in pluripotent cells and assess its role in ectoderm differentiation, as well as its effects on LHX5 expression and DNA-binding activity.



2. Ongoing work aims to characterize germ layer-specific H2H lncRNAs by examining:

- Their enhancer-hosting capacity
- The regulatory mechanisms by which lncRNAs fine-tune the activity of lineage-specific transcription factors.

Expression profiling of H2H TF-lncRNA pairs across the three primary germ layers

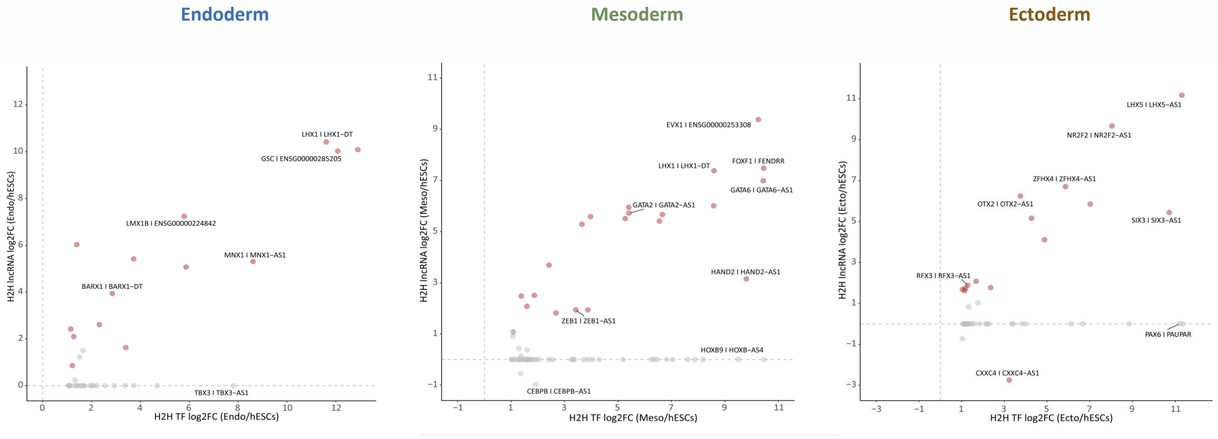


- Shared promoters of TF-lncRNA in H2H orientation are characterized by highly accessible chromatin.

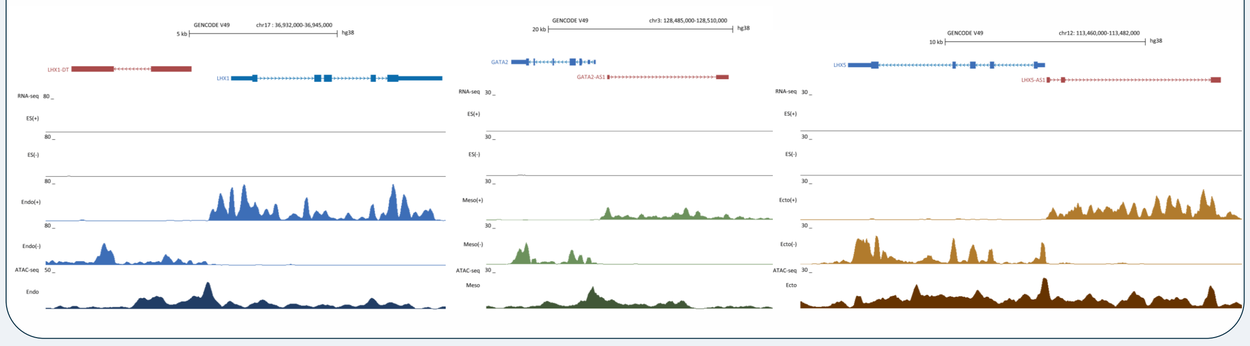
Differentially expressed lncRNAs (p-value ≤ 0.05)

Non. diff expressed lncRNAs (p-value ≥ 0.05)

Endoderm Mesoderm Ectoderm

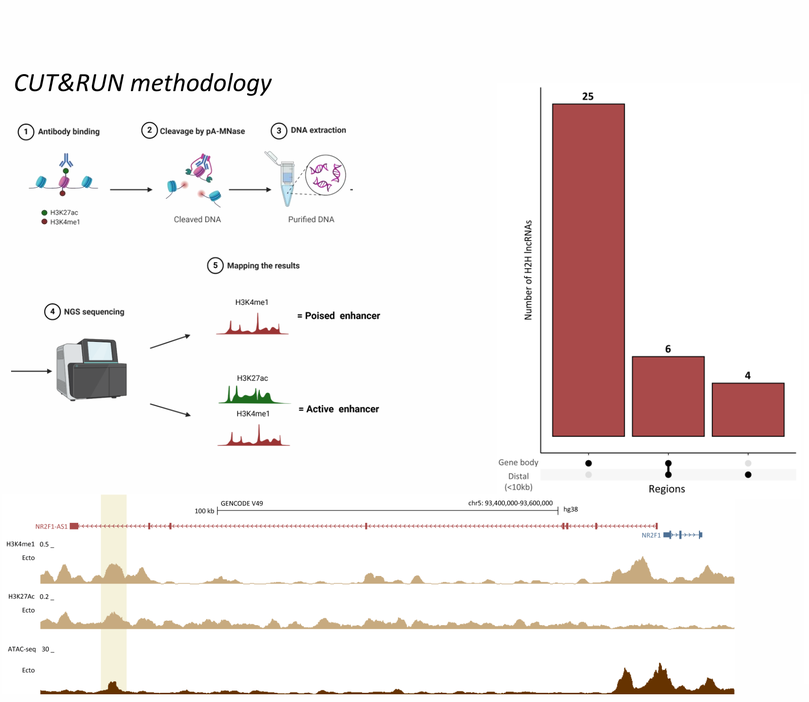


- Many H2H lncRNAs show lineage-specific high expression patterns similar to their neighboring TFs.



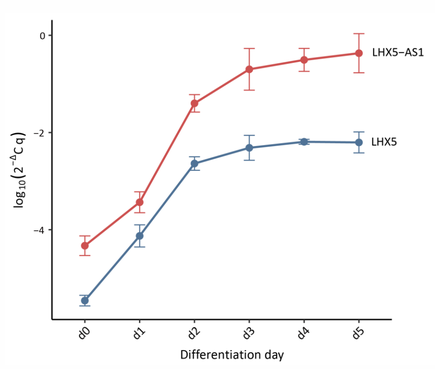
Detecting enhancer regions in primary germ layers

CUT&RUN methodology



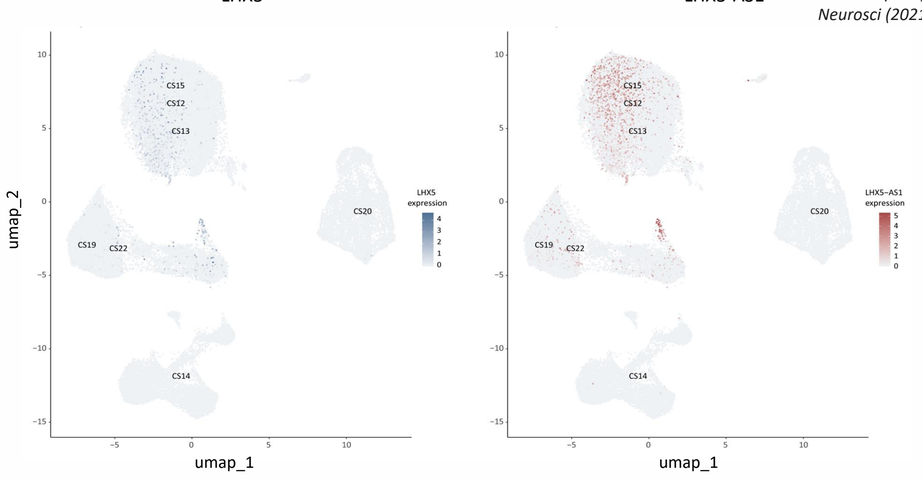
- We identified 35 H2H-oriented lncRNAs that host lineage-specific putative enhancers.

LHX5 and LHX5-AS1 expression during ectoderm differentiation and early human brain development



- LHX5 and LHX5-AS1 share a common expression profile during ectoderm differentiation.

Data adapted from: Eze et al., Nat Neurosci (2021)



- LHX5 and LHX5-AS1 display overlapping expression across brain developmental stages.